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SELECTIVE EXTRACTION AND FRACTIONATION OF NATURAL PHOSPHOLIPID MIXTURES BY SUPERCRITICAL CO_2 AND COSOLVENT MIXTURES

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Summary

Phospholipid (PL) mixtures from natural sources, such as vegetable seeds, are valuable products that find widespread use throughout several industries. Traditionally, PLs have been obtained from a by-product associated with the seed oil processing industry, i.e., lecithin from the soybean oil refining process. Supercritical carbon dioxide (SC-CO_2) extraction of seed oils leaves much of the PL content of the seeds in the deoiled meal, whereas in the traditional refining process, the PLs are partitioned into the hexane-extracted oil. To optimize and enhance the value of a previously developed supercritical fluids (SF) process for removing oil from soybean flakes, we have devised a two step, sequential scheme for the extraction of both oil and PL-containing fractions using SC-CO_2 and SC-CO_2/ethanol mixtures. Experimental data indicate that the PLs are selectively removed from the flakes using the SC-CO_2/ethanol mixture. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are more readily solubilized in the SC-CO_2/cosolvent mixtures than phosphatidylinositol (PI). The extent of recovery of PC and PE is a function of the molar fraction of cosolvent in the SC-CO_2. Selective fractionation of PC with respect to PE can be affected by varying the molar fraction of ethanol dissolved in the SC-CO_2. The extracts from the SF process were characterized by inductively coupled plasma spectroscopy (ICP) and high performance liquid chromatography/evaporative light scattering detection (HPLC/ELSD) analysis, for total phosphorus and individual PL content, respectively. A thorough mass balance of the process will also be presented.

1. INTRODUCTION

Phospholipids (PLs) are polar conjugate lipids [1,2]. The molecular structure of the PLs commonly occurring in plants are shown in Figure 1 [3-7]. The terms lecithin and PC are often used interchangeably [2]. However, the term lecithin refers commercially to a complex naturally occurring mixture of PLs, traditionally obtained by water-washing crude vegetable oil and separating and drying the hydrated gums [3]. Therefore, the term lecithin is often used to describe a diverse group of commercially available PL mixtures, including fractions containing one or more PLs, triglycerides, pigments, carbohydrates, sterols, cerebrosides, in different proportions [3,4].

Earlier studies conducted at the National Center for Agricultural Research in Peoria, Illinois, (USA), by Friedrich and coworkers [8,9] showed that supercritical carbon dioxide (SC-CO_2) was very effective in removing oils from a variety of seed matrices, devoid of any appreciable PL content. This property has recently been exploited by List, et al [10]

to continuously degum pre-extracted soybean oil using SC-CO_2. However, the limited solubility of PLs in SC-CO_2 leaves behind a potentially valuable by-product in the spent seed matrix that could be recovered to economic advantage. In addition, any PL recovery process must also be compatible with the end use of the seed protein meals as an animal feed, or for use in human food consumption.

The recovery of the lecithin components from a seed matrix must also take into consideration the effect of the recovery process on the environment as well as regulatory aspects, such as food safety. For this reason, the SFE process is particularly appropriate, since the chosen extracting agent is both environmentally acceptable and non-toxic to food consumers. Since neat CO_2 will not effectively solubilize PL moieties, the choice of a suitable cosolvent to enhance their solubility must be made not only on a thermodynamic basis, but also with regard to its food safety status as: "Generally Recognized As Safe" (GRAS).

A logical choice for a cosolvent is ethanol, which enjoys GRAS status in the United States. Ethanol has been previously used to fractionate PLs [11], although not in a SFE process; however it has been utilized by Temelli [12] to qualitatively demonstrate the removal of phospholipids from canola seed using SC-CO_2. Since high pressure phase equilibria is available for ethanol/CO_2 mixtures [13], our efforts have focused on the use of this cosolvent in fractionating the PL mixtures.

The objectives of this research are: a) to develop an integrated procedure for the removal of oil and phospholipid fractions from seed matrices by using supercritical fluids; b) by use of an appropriate cosolvent, to solubilize the lecithin fraction into the SC-CO_2/cosolvent mixture and to further study the fractionation of PLs under supercritical conditions.

II. EXPERIMENTAL PROCEDURE

The soybeans were provided by Illinois Crop Improvement Association, Inc. (Williams 82 Soybeans, Lot 4849, Producer # 14290).

The seeds were found to have a oil content of 20.52 weight %, their moisture content was 10.84 wt. %, and total phosphorus content was 5.134 mg/g.

The experimental extractions were performed with the apparatus shown in Figure 2. This unit is a modification of an apparatus previously used in our laboratory [14], but modified to permit cosolvent addition. The CO_2 was delivered by a Haskel gas booster pump (Model AGT 62-152, Haskel, Inc., Burbank, CA) through a check valve to a two-way switching valve (SV1/SV2). From SV1 it enters a three-way tee where ethanol can be added for the second stage extraction via an HPLC pump (Model 100A, Beckman Instrument, Inc., Fullerton, CA). The CO_2 or CO_2/cosolvent mixture then passes through an equilibration coil (EC2) inside a temperature controlled GC oven. Both extraction fluids pass downward through the soybean flakes contained in the extraction vessel, exiting at the bottom of the extractor and flowing through another switching valve (SV3/SV4). From here the dissolved extract passes through a heated micrometering valve (Series

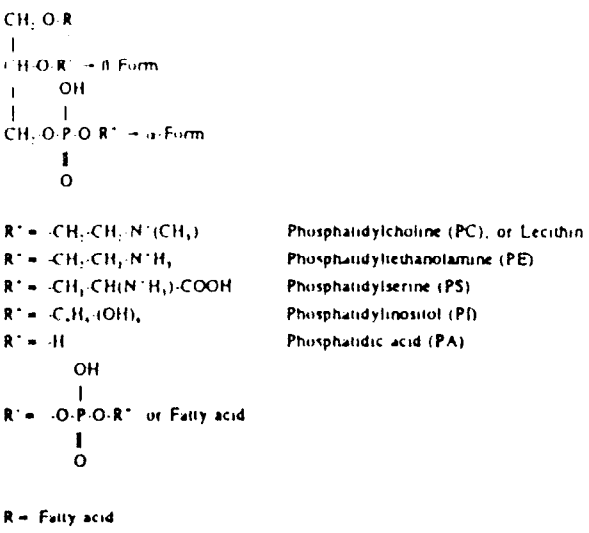


Figure 1 : Commonly occuring phospholipids in plants.

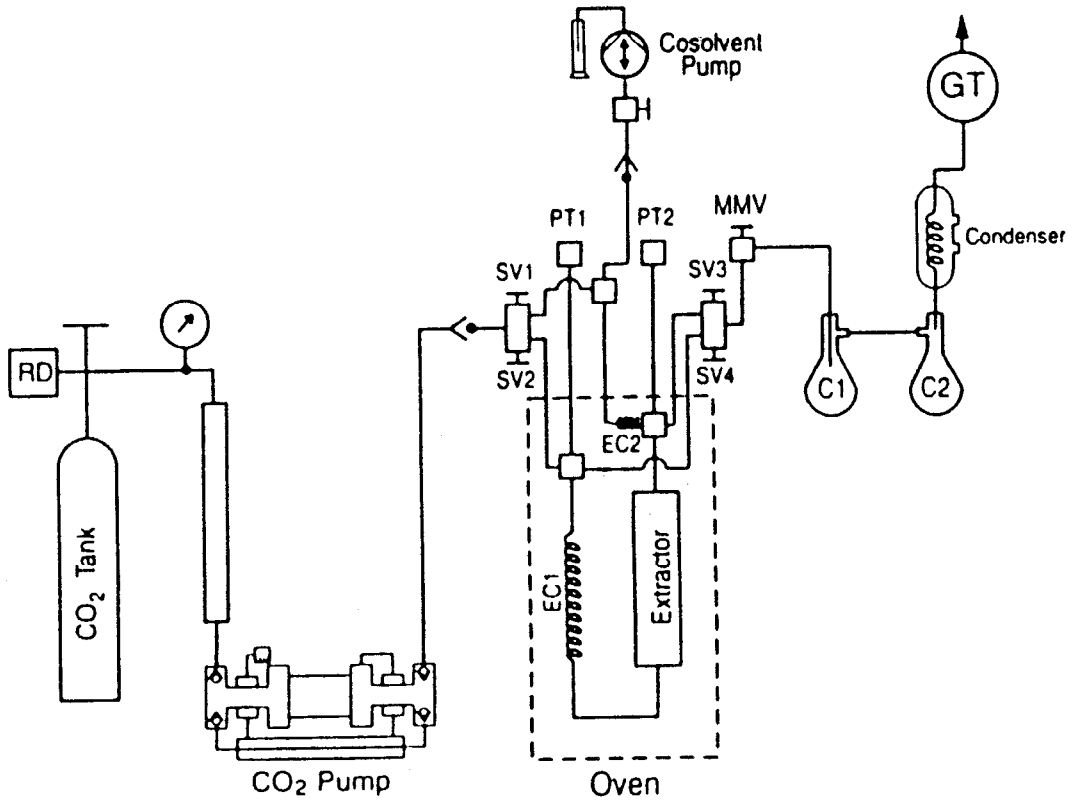
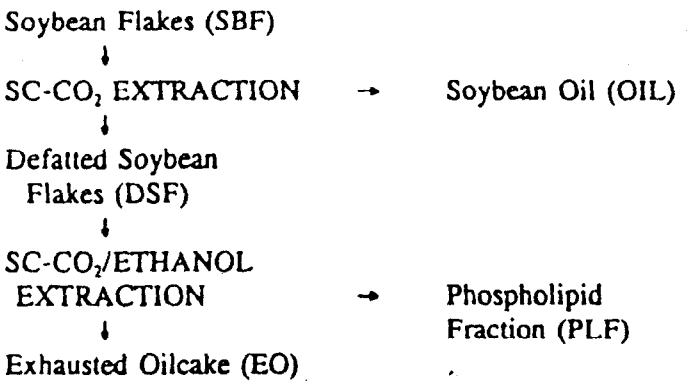


Figure 2 : Supercritical fluid extraction system with cosolvent addition.

30VRMM, Autoclave Engineers, Erie, PA) into the first of two collection flasks (C1). Ethanol entrained in the decompressed CO_2 stream is further separated with the aid of a second flask (C2) and a jacketed, cool condenser. Total and intermediate volumes of CO_2 were ascertained using a gas totalizer (Model DMT200A, American Meter Division, Philadelphia, PA).

The described experiments were conducted on samples of approximately 60 g; the seeds were ground and thinly flaked (0.1-0.25 mm), in accordance with the findings of Snyder, et al [8].

The extractions were carried out using the following two extraction steps:



The initial SC-CO_2 extraction was performed at 10,000 PSI (70 MPa) and 80°C; optimal conditions for the extraction of seed oils [8,9]. The second extraction was performed at 9,900 PSI (68.2 MPa), 80°C, always using 80 ml of ethanol (63.2 g). The ethanol molar fraction (X_{EtOH} = 0.05-0.20) was changed by altering the ethanol flow rate (0.44-2.67 ml/min) and/or the CO_2 flow rate (1.60-4.00 l/min).

To develop a mass balance flow-sheet for the extractions, the following analyses were performed for SBF, OIL, PLF, and EO: total weight, dry matter [15], crude fat [16], and total phosphorus. To determine the total phosphorus, the samples were ashed [17] and then dissolved in 5 % HNO_3. Phosphorus content was determined by ICP (Model 400, Plasma Emission Spectrometer, Perkin Elmer, Norwalk, CT).

HPLC analyses were performed using the method of Abidi [18] and Mounts, et al [19]. PLFs were dried and than redissolved in 3 ml of chloroform before HPLC analysis. PLs were extracted from SBF, EO, and DSF by a three-fold extraction using the extraction solvent mixture of Bollomann [20, 21], then dried, and redissolved in chloroform.

III. RESULTS

Table 1 tabulates the mass balance for three extractions using different mole fractions of ethanol. They have been normalized to 1 Kg of SBF for the neat SC-CO_2 extraction, and different ethanol cosolvent additions for the second extraction (X_{EtOH} = 0.052, 0.102, and 0.167, respectively). As noted in Table 1, there is a insignificant discrepancy between the

$X_{e_2} = 0.052$

	IN					OUT				
	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg
SBF	1000	892	205	108	5434					
OIL						239	200	200	39	3
PLF						2	2	2	--	43
EO						692	674	4	18	5394
Total	1000	892	205	108	5434	933	876	206	57	5440
Δ in g						- 67	- 16	+ 1	- 51	+ 6
Δ in %						- 6.7	+ 1.8	+ 0.5	- 47	+ 0.1

$X_{e_2} = 0.102$

	IN					OUT				
	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg
SBF	1000	892	205	108	5434					
OIL						217	198	198	19	12
PLF						8	8	8	--	365
EO						688	668	1	20	5157
Total	1000	892	205	108	5434	913	874	207	39	5534
Δ in g						- 87	- 18	+ 2	- 69	+ 100
Δ in %						- 8.7	- 2.0	+ 1.0	- 64	+ 1.8

$X_{e_2} = 0.167$

	IN					OUT				
	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg	W.M. g	D.M. g	FAT g	WATER g	PHOSPHORUS mg
SBF	1000	892	205	108	5434					
OIL						209	188	188	21	39
PLF						11	11	11	--	314
EO						707	685	1	22	5016
Total	1000	892	205	108	5434	927	884	200	43	5369
Δ in g						- 73	- 8	- 5	- 65	- 65
Δ in %						- 7.3	- 0.9	- 2.4	- 60	- 1.2

Table 1: Mass Balance Flow Sheet for Consecutive Extractions at Different Ethanol Molar Fractions.

IN and OUT mass balances, confirming the success of these experiments. The high Δs for the Wet Matter (W.M.) are due to water loss via entrainment in the CO_2. Hence the mass balances for water are in error, due to its loss from the receiver. However, the mass balances of the Dry Matter (D.M.) are very acceptable. Further confirmation comes from comparing the Δs for the D.M. and the difference in Δs between W.M. and Water. Here Δs for the W.M. minus the Δs for the corresponding Water equal the Δs for the D.M.. The mass balances for the oil and phosphorus content are impressive, and indicate that the extractions can be well characterized. In fact, the average % error in the mass balance for the phosphorus, oil, and D.M. are 1.0 , 1.3, and 1.6 %, respectively.

The total phosphorus can be converted to a total PL basis by using a conversion factor of 30 [22]. Figure 3 shows the total amount of PLs recovered from each of the above extractions by varying the amount of ethanol in the second extraction. At X_{EtOH} = 0.052 the recovery is very low, as shown in Figure 3, however PL recoveries can be increased considerably using mole fractions of ethanol corresponding to 0.102 and 0.162, respectively.

HPLC results of the PL content of PLF and EO fractions are shown in Figure 4. The data indicate that PC and PE are generally extracted in preference to PI. Note that the quota of ethanol greatly influences the total amount of all the PLs extracted, and that at least a X_{EtOH} of 0.150 is needed to optimize the extraction of both PE and PC. The amount of ethanol in the SC-CO_2 also influences the composition of the resultant PLF. For example, using X_{EtOH} greater than 0.102 one can vary the relative amounts of PC to PE in the resultant extracts. A small amount of ethanol (5%) in the SC-CO_2 was not sufficient enough to extract the PLs. Phosphatidic acid, a minor PL component of soybean, was not solubilized to any great extent in the SC-CO_2/ethanol mixtures.

IV. CONCLUSION

The above studies show that a consecutive, two step SFE process can be used to deoil vegetable seeds as well as isolate phospholipid-enriched fractions. The former can be accomplished using neat SC-CO_2 extraction followed by a SC-CO_2/ethanol extraction for selective PL removal. The molecular specificity of the SC-CO_2/ethanol extractions for specific PLs is similar to results using alcoholic liquid solvent extraction. The described method has the possibility of producing industrially-useful PL mixtures varying in PC and PE content. Scaled up experiments on a 4-liter pilot plant are currently being conducted.

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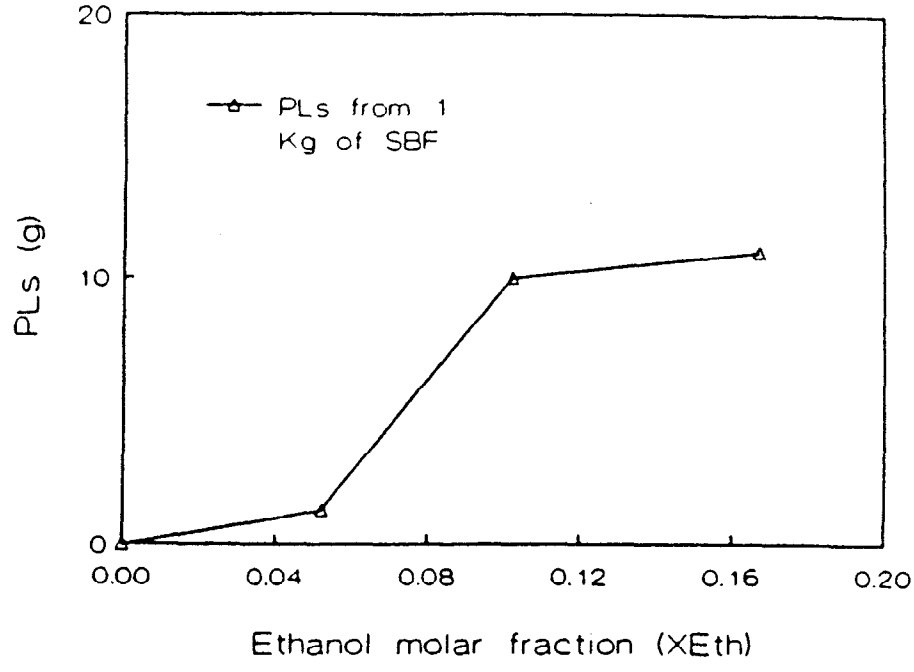

Figure 3 : Phospholipid yield per Kg of soybean flakes as a function of ethanol concentration in the supercritical phase.

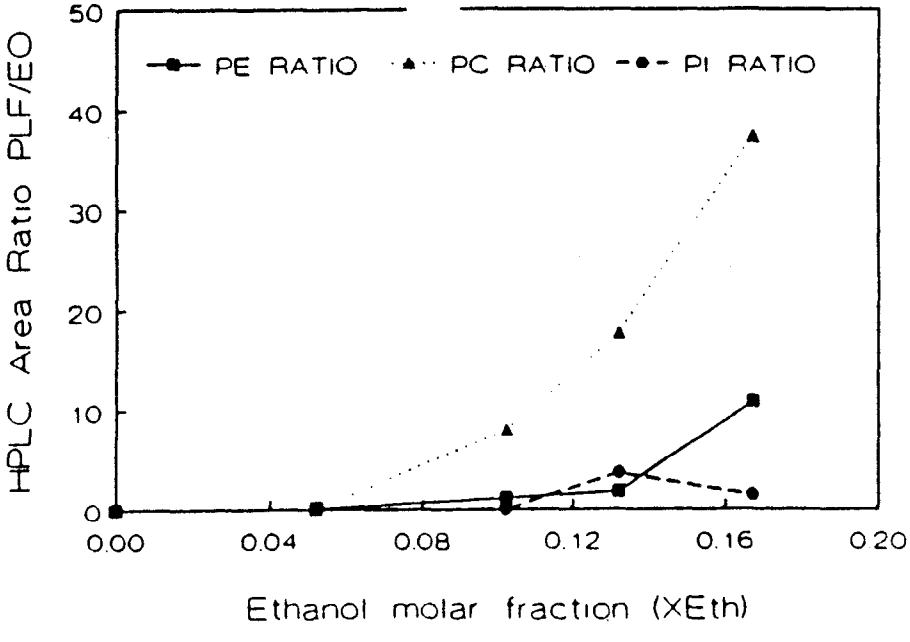

Figure 4 : PLF/EO ratio of PE, PC, and PI, using different ethanol molar fractions in SC-CO_2.

DISCLAIMER

The mention of firm names does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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